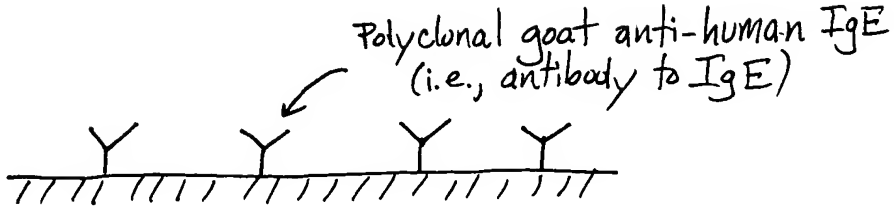
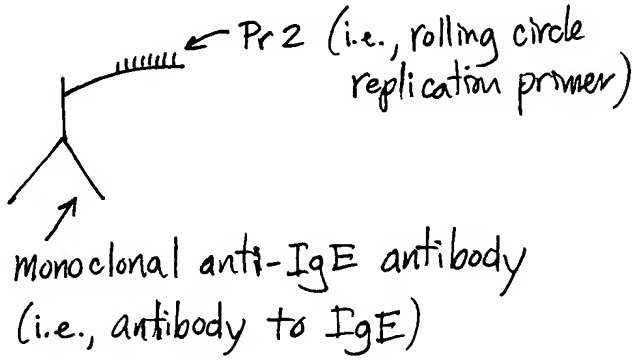
Appendix A
Kingsmore et al. Example 7

Materials:

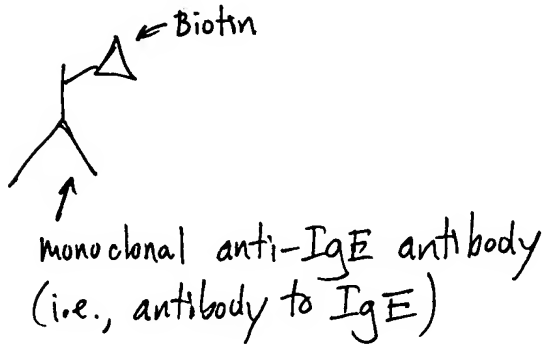
ⓐ Microarray:
(lines 4-8)



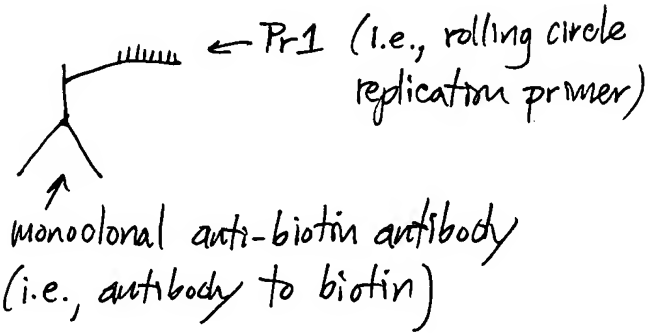
ⓑ First conjugate:
(Pr2~Mab Anti-IgE)
(lines 8-12)



ⓒ Second conjugate:
(biotin~Mab Anti-IgE)
(lines 8-9 & 12-13)



ⓓ Mab Anti-biotin conjugate:
(lines 14-18)



ⓔ Circle 1 (for Pr1): (1)
(lines 24-25)

ⓕ Circle 2 (for Pr2): (2)
(lines 25)

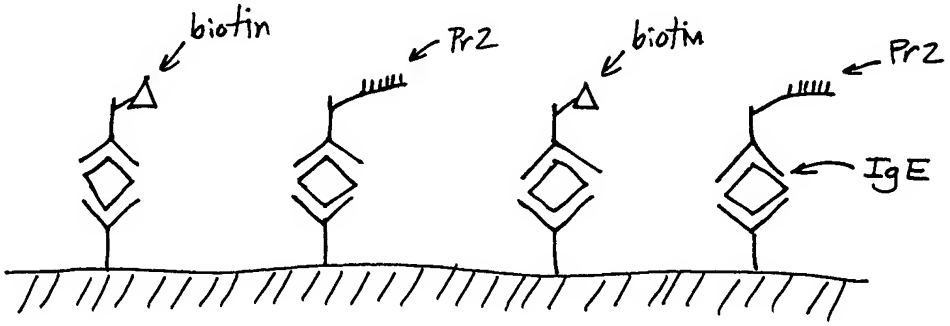
Method

① Antigen-antibody complexes pre-formed in separate reactions (lines 18-21)

biotin~Mab Anti-IgE ⊕ IgE in one tube

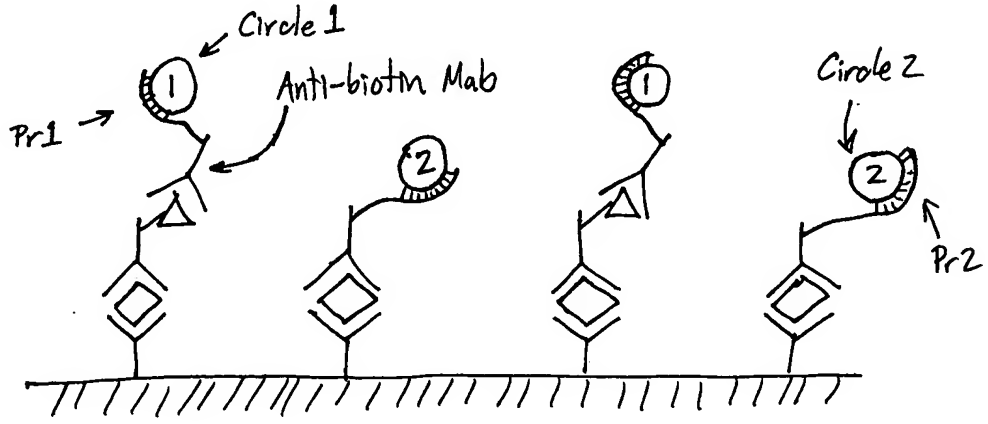
Pr2-Mab Anti-IgE ⊕ IgE in a second tube

② Mix antigen-antibody complexes and apply mixtures to arrays (lines 21-24)



③ Apply mixture of Circle 1, Circle 2 and Pr1~Mab Anti-biotin to microarrays (lines 24-27)

Note: Circles now associated with antibody-primer conjugates

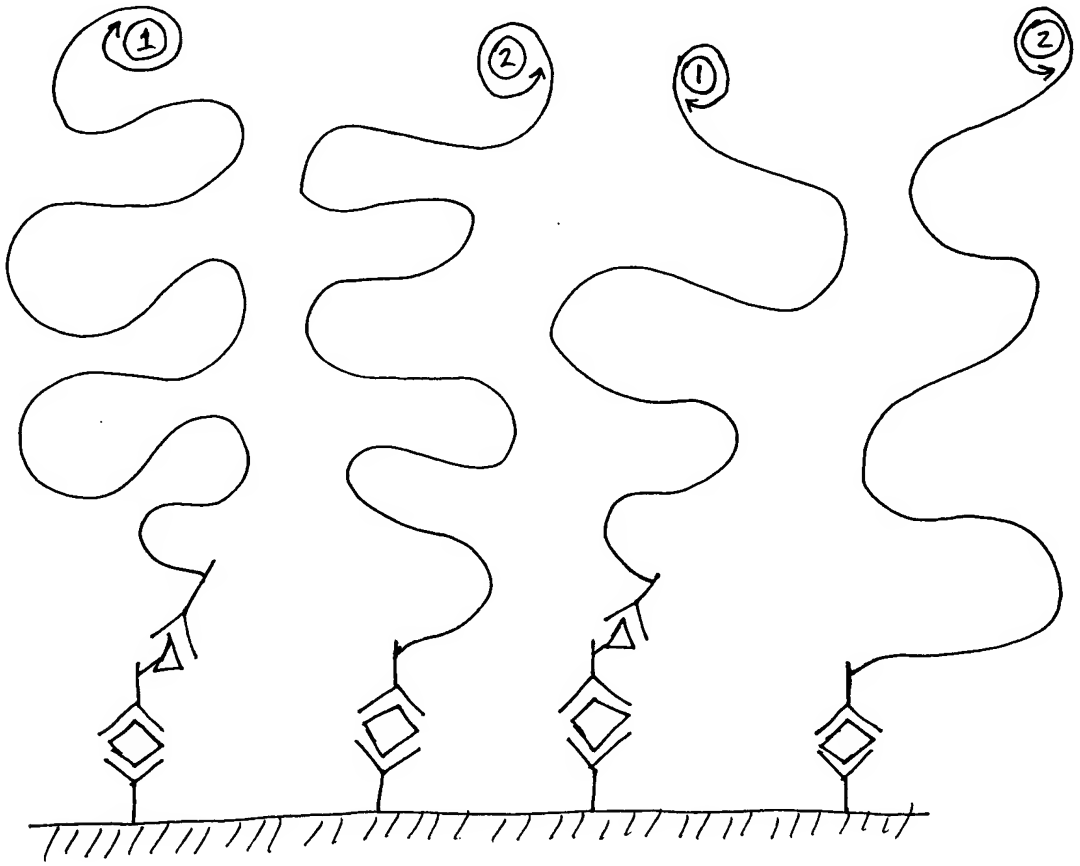


④ Wash slides [to remove unbound components] (lines 27-28)

Note: Circles associated with antibody-primer conjugates are not dissociated in this step (otherwise, rolling circle amplification would not take place)

⑤ Apply RCA reaction mix (buffer, labeled detector probes, polymerase, dNTPs) and incubate [rolling circle amplification of Circle 1 and Circle 2 primed from Pr1 and Pr2, respectively] (lines 28-33)

Note: Circles are still associated with antibody-primer conjugates



⑥ Wash, dry and scan slides [to detect labeled probes]